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COMMENTARY

A Radical Concept on Caveolae and Endothelial Dysfunction in Coronary Microvascular Disease in Diabetes

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The risk of cardiovascular disease is markedly elevated in individuals with diabetes, representing the primary cause of morbidity and mortality in these diabetic patients (1). More specifically, individuals with diabetes display dysfunctions in the regulation of blood flow in coronary arterioles (2,3). Importantly, impairments in the appropriate vasodilatory response of myocardial arterioles to various pharmaceutical and physical stimuli can be present even if there is no discernible atherosclerotic blockage in these blood vessels (4,5). Moreover, increased flow-mediated dilation (FMD) in coronary arterioles is an important regulatory mechanism for controlling arteriolar diameter and blood flow in response to changes in wall shear stress, and this mechanism is also impaired in conditions of glucose dysregulation (6,7).

While the underlying etiology for the dysfunctions in the coronary microcirculation in diabetes is certainly multifactorial, the contribution of impairments in the endothelial nitric oxide (NO)–generating system and their association with the excess generation of reactive oxygen species (ROS) appears to be crucial in the development of these vascular abnormalities. For example, the impairment of the induction of vasodilation in coronary arterioles in the *db/db* mouse, a model of obesity-associated insulin resistance and type 2 diabetes, is related to limitations in NO availability (8,9). It is of great interest that NO availability can be compromised by interactions with superoxide anion, with a by-product of this reaction being the generation of peroxynitrite ($OONO^-$), an ROS that itself is known to mediate deleterious effects on the cardiovascular system in diabetes (10). However, prior to the study of Cassuto et al. (11), which appears in this issue, the specific impact of $OONO^-$ on the endothelial caveolae, which is required for the proper functionality of endothelial NO synthase (eNOS) (12), and the subsequent effect of this interaction on NO-regulated FMD in response to changes in wall shear stress in conditions of human diabetes had not been rigorously addressed in the scientific literature.

The study of Cassuto et al. (11) convincingly advances the concept that, in human diabetes, defects in the ability of NO to facilitate vasodilation in coronary arterioles under conditions of increased $OONO^-$ exposure are related to the impaired expression of the caveloin-1 (Cav-1), an important structural component of caveolae in the endothelial membrane. Using tissue isolated from older nondiabetic subjects and subjects with either type 1 or 2 diabetes, the authors demonstrated that in vitro increases in FMD due to enhanced wall shear stress were severely reduced in coronary arterioles from diabetic subjects compared with coronary arterioles from nondiabetic subjects. Moreover, these vasomotor defects were associated with augmented $OONO^-$ production, as reflected by 3-nitrotyrosine levels (a biomarker of $OONO^-$-mediated protein nitration), and were reproduced by direct incubation of arterioles with $OONO^-$. A critical finding was that protein expression of membrane-localized Cav-1, a critical component of caveolae, was significantly reduced in the diabetic group, a result reproduced in primary cultured human coronary artery endothelial cells exposed to high glucose (25 mmol/L) for 24 h, and colocalized with the 3-nitrotyrosine. In addition, pharmacological disruption of caveolae in nondiabetic arterioles significantly reduced FMD. Finally, the association of the disruption of caveolae assembly with uncoupling of eNOS in the diabetic group was convincingly demonstrated using isolated coronary arterioles from Cav-1 knockout mice, in which endothelial caveolae are completely absent. Importantly, the marked defects in FMD in response to wall shear stress were reversed by the addition of sepiapterin, a stable precursor of

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the NOS cofactor BH_4, in an NO-dependent fashion. Overall, these impairments in vasomotor regulation of coronary arterioles and FMD in diabetes likely contribute to an overall increase in the risk of coronary microvascular disease. These major findings and conclusions are summarized in Fig. 1.

Cassuto et al. (11) have provided a comprehensive in vitro evaluation of the relationships among $OONO^-$ levels, caveolae assembly in endothelial membranes, eNOS functionality, and FMD in coronary arterioles from diabetic human subjects, using sound and reliable pharmacologic and genetic approaches. Therefore, there is clear clinical relevance of the findings to increasing our understanding of the etiology of coronary microcirculatory disease in human diabetes. However, there are some limitations that should be noted. The mixing of individuals with type 1 and 2 diabetes into a single subject pool is less than optimal, and the very limited number of subjects with type 1 diabetes (n = 2) makes application of these findings to that specific condition difficult. There is a markedly unequal distribution of male and female subjects in the investigation. There is no description of the oral antidiabetic and antihypertensive medications the diabetic subjects were taking, some of which are certainly vasomodulatory compounds that could potentially confound the results (13). Finally, the discussion, while being quite informative, could have been improved by providing at least some information on the etiology of free radical production in conditions of type 1 and 2 diabetes that contribute to the generation of $OONO^-$, including the role of nutrient overload (elevations in both glucose and lipid) leading to mitochondrial overactivity/dysfunction and excess H_2O_2 emission (14), overactivation of NADPH oxidase (15,16), and eNOS uncoupling (12,17).

The findings of Cassuto et al. (11) provide important new information regarding the underlying cellular mechanisms responsible for vasomotor dysfunctions in coronary arterioles in diabetic humans. These results could be used as the basis for the design of interventions to improve coronary blood flow and cardiac function in diabetes by the prevention of free radical overproduction and sequestration of free radicals, although this topic remains controversial (3,12). An intriguing additional application of the findings of Cassuto et al. (11) is to assess whether these endothelial dysfunctions in feed arterioles also exist in skeletal muscle tissue in human diabetes, especially in type 2 diabetes, as NO-regulated blood flow to this tissue has a major impact on the delivery of glucose, insulin, and other factors to skeletal muscle, and plays an important role in whole-body glucoregulation (18,19).

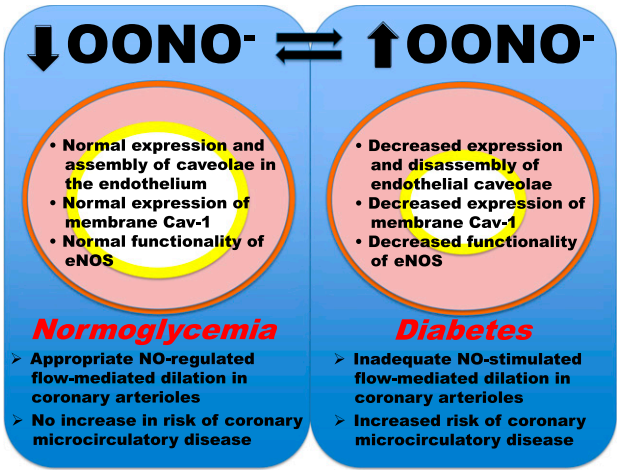

Figure 1—The impact of $OONO^-$ on the regulation of coronary microcirculation in conditions of diabetes. The figure illustrates the key concepts of the study of Cassuto et al. (11), indicating how elevated $OONO^-$ levels can impair the expression and proper assembly of endothelial caveolae, leading to dysregulation of NO-dependent FMD in coronary arterioles, thereby increasing the risk of coronary microcirculatory disease in humans with type 1 or 2 diabetes.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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